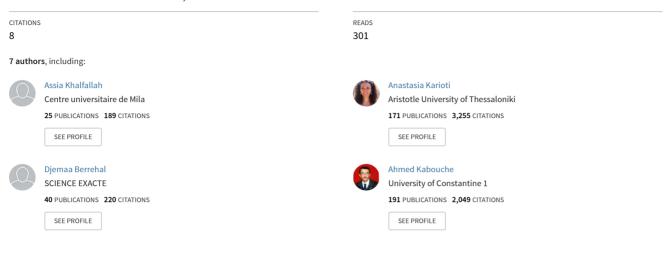
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# A New Flavonol Triglycoside and Other Flavonol Glycosides from Astragalus armatus Willd. (Fabaceae)

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**Abstract:** From the aerial parts of *Astragalus armatus*, a new acylated flavonol triglycoside, isorhamnetin-3-O-(5<sup>III</sup>-p-hydroxybenzoyl)- $\beta$ -apiofuranosyl-(1 $\rightarrow$ 2)[ $\alpha$ -rhamnopyranosyl-(1 $\rightarrow$ 6)]- $\beta$ -galactopyranoside (1) which we named astrarmatuside, has been isolated and structurally elucidated together with seven known flavonol glycosides: tamarixetin-3-O- $\alpha$ -apiofuranosyl-(1 $\rightarrow$ 2)[ $\alpha$ -rhamnopyranosyl-(1 $\rightarrow$ 6)]- $\beta$ -galactopyranoside (2) (millettiaspecoside D), isorhamnetin-3-O- $\beta$ -apiofuranosyl-(1 $\rightarrow$ 2)[ $\alpha$ -rhamnopyranosyl-(1 $\rightarrow$ 6)]- $\beta$ -galactopyranoside (3), kaempferol-3-O- $\alpha$ -rhamnopyranosyl-(1 $\rightarrow$ 2)[ $\alpha$ -rhamnopyranosyl-(1 $\rightarrow$ 6)]- $\beta$ -galactopyranoside (4), kaempferol-3-O- $\alpha$ -rhamnopyranosyl-(1 $\rightarrow$ 2)[ $\alpha$ -rhamnopyranosyl-(1 $\rightarrow$ 6)]- $\beta$ -galactopyranoside (mauritianin) (5), isorhamnetin-3-O- $\alpha$ -rhamnopyranosyl-(1 $\rightarrow$ 2)[ $\alpha$ -rhamnopyranosyl-(1 $\rightarrow$ 6)]- $\beta$ -galactopyranoside (6), kaempferol-3-O- $\alpha$ -rhamnopyranosyl-(1 $\rightarrow$ 2)[ $\alpha$ -rhamnopyranosyl-(1 $\rightarrow$ 6)]- $\beta$ -galactopyranoside (6), kaempferol-3-O- $\alpha$ -rhamnopyranosyl-(1 $\rightarrow$ 2)[ $\alpha$ -rhamnopyranosyl-(1 $\rightarrow$ 6)]- $\beta$ -galactopyranoside (6), kaempferol-3-O- $\alpha$ -rhamnopyranosyl-(1 $\rightarrow$ 2)[ $\alpha$ -rhamnopyranosyl-(1 $\rightarrow$ 6)]- $\beta$ -galactopyranoside (6), kaempferol-3-O- $\alpha$ -rhamnopyranosyl-(1 $\rightarrow$ 2)[ $\alpha$ -rhamnopyranosyl-(1 $\rightarrow$ 6)]- $\beta$ -galactopyranoside (6), kaempferol-3-O- $\alpha$ -rhamnopyranosyl-(1 $\rightarrow$ 6)]- $\beta$ -glucopyranoside (narcissin) (8). The structures of the isolated compounds were established by means of 2D NMR experiments, HPLC-DADMS, HR-MS and UV spectral analyses. Pivotal role in the structure elucidation and in particular in the determination of sugar sequence, played HSQC-TOCSY and ROESY experiments whereas those of the known compounds (2-8) were established by spectral comparison with those published in the literature.

**Keywords:** *Astragalus armatus*; Fabaceae; Acylated flavonol triglycoside; HSQC-TOCSY; ROESY. © 2014 ACG Publications. All rights reserved.

## 1. Introduction

Astragalus (As-trá-ga-lus), belonging to the legume family Fabaceae, subfamily Faboideae, is a large genus of about 2000 species and is considered to be the largest and most complex genus in Angiosperm [1,2]. It is distributed primarily in cold, arid and semiarid mountainous regions of the Northern Hemisphere (Europe, Asia, N. America) and South America. In North Africa, some species are used against cough, asthma, arthritis and scorpions' stings [3]. A. armatus Willd. is an endemic

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shrub of the Northern Africa (Algeria, Morocco, Tunisia) [4], distributed in the pre-Saharian zone and is associated with the desertification in arid areas due to overgrazing [5]. In Tunisia, it is used as tonic, stimulant and in cases of anaemia [6]. In Algeria, plants belonging to the genus are used in cases of fatigue, numbing, cold, flu, asthma, arthritic pains and deficiency of the immunitary system [7]. The purported health benefits of *Astragalus* are a bit vague, at least from a scientific standpoint. *Astragalus* prevent asthma and allergy symptoms, it has been proposed as an herbal remedy for everything from AIDS, chronic fatigue syndrome, hepatitis, and myasthenia gravis to cancer [8,9]. It is almost always combined with other herbs, complicating the evaluation of the health benefits of *Astragalus*. In continuation of our works on Fabaceae family [10], we report here the isolation and structure elucidation of a new acylated flavonol triglycoside (astrarmatuside) together with seven known flavonol glycosides from the endemic species *Astragalus armatus* Willd.

## 2. Materials and Methods

#### 2.1. General

UV spectra were recorded on a Agilent 8453 UV-Visible spectrophotometer, in MeOH. HR-ESI MS mass spectra were measured on an Ionspec Ultima FTMS spectrometer using 2,5-dihydroxybenzoic acid (DHB) as matrix.

Column chromatography (CC): Polyamide (ICN Biomedicals GMBH 09602) and silica gel 60 (Merck, Art. 9385), gradient elution with the solvents mixtures indicated in each case;. TLC: Polyamide (ICN Biomedicals GMBH 09603), Merck silica gel 60  $F_{254}$  (Art. 5554); Detection: UV-light, spray reagent (vanillin-H<sub>2</sub>SO<sub>4</sub> on silica gel).

#### 2.2. Plant material

Aerial parts of *Astragalus armatus* were collected from Bekira-Constantine (Eastern Algerian) in May 2007. Voucher specimen has been kept in the Herbarium of the faculty of sciences (University of Constantine 1) under the number LOST.Aa.05.07.

#### 2.3. Extraction and isolation

Air-dried powdered aerial parts of Astragalus armatus (1.5 kg) were macerated four times with 70% EtOH solution. The hydro-alcoholic solution was concentrated under reduced pressure to dryness and the residue was dissolved in water and kept in overnight. After filtration, the aqueous solution was successively extracted with CH<sub>2</sub>Cl<sub>2</sub>, EtOAc and *n*-BuOH for three times with each solvent, then the EtOAc and *n*-BuOH extracts were concentrated to dryness. The *n*-BuOH extract (25 g) was subjected to a MN-SC6 polyamide column chromatography being eluted with a gradient system of toluene/MeOH with increasing polarity. Four main fractions (A-D) were collected. Fraction A (210 mg) was further purified by CC silica gel (EtOAc:MeOH:H<sub>2</sub>O 10:2:1) and yielded a yellow precipitate (100 mg) which was identified as compound 8. Fraction B (100 mg) was subjected to CC over silica gel using EtOAc:MeOH:H<sub>2</sub>O 10:2:1 and afforded impure compound **2**, which was further separated by TLC on silica gel (4.5 mg) using the same solvent system. CC over silica gel of fraction C (150 mg) afforded compound 3 (5.0 mg) which was further purified on polyamide TLC eluted with  $(H_2O:MeOH:butanone:acetic acid 13:3:3:1)$  and a mixture (4.0 mg) of compounds 5 and 6, whereas fractionation of D (250 mg) under the same chromatographic conditions led to three subfractions: D1-D3. D1 (80 mg) was subjected to polyamide TLC (MeOH:H<sub>2</sub>O:acetic acid, 18:1:1) and yielded compounds 1 (3.9 mg) and 7 (2.0 mg). D2 (100 mg) was purified by silica gel TLC (EtOAc:MeOH:H<sub>2</sub>O 10:2:1) to give compound 4 (7.0 mg) and D3 (90 mg) was purified on polyamide TLC using (H<sub>2</sub>O:MeOH:butanone:acetic acid 13:3:3:1) leading to 5 (3.0 mg).

#### 3. Results and Discussion

From the methanolic extract of the aerial parts of Astragalus armatus eight flavonol glycosides (1-8) were isolated (Fig. 1). Compound 1 (astrarmatuside) was obtained as an amorphous yellowish powder. isorhamnetin-3-O-(5<sup>'''</sup>-p-hydroxybenzoyl)- $\beta$ -apiofuranosyl-(1 $\rightarrow$ 2)[ $\alpha$ -It was identified as rhamnopyranosyl- $(1\rightarrow 6)$ ]- $\beta$ -galactopyranoside by 1D, 2D NMR and HPLC-UV-DAD-MS analyses and by MS spectrometry. Its HPLC-UV-DAD data showed the presence of a flavonol derivative (255, 266sh, 330 nm) while its MS spectra exhibited pseudomolecular ions  $[M+Na]^+$  at m/z 899.7, and  $[M-Na]^+$ H] at m/z 875.7 in the positive and negative ion mode, respectively. This suggested a molecular weight of 876, compatible with the molecular formula  $C_{40}H_{44}O_{22}$ . High resolution ESI-MS spectra in the negative ion mode were in agreement, as they exhibited a pseudomolecular peak at  $[M-H]^{-} m/z$ 875.22384 (theoretical 875.22405) corresponding to a molecular formula  $C_{40}H_{43}O_{22}$ . In agreement, 40 carbon signals were observed in the <sup>13</sup>C NMR (SEFT) spectrum.<sup>1</sup>H NMR and <sup>13</sup>C NMR spectra of 1 showed characteristic shift values and multiplicities of a 3-O- $\beta$ -glycosylated isorhamnetin derivative [11]. In the aromatic area of the proton spectrum an ABX system was evident which was attributed to the protons H-2', H-6', and H-5' (at  $\delta_{\rm H}$  8.04, d, J = 1.9; 7.36 dd, J = 8.4, 2.0; and 6.90 d, J = 8.4, respectively) of the flavonoid nucleus. Protons H-6 and H-8 were resonating as doublets below 6.5 ppm (at  $\delta_{\rm H}$  6.08 and 6.17), indicating that the glycosylation site was other than position 7. Besides the 15 carbon signals of the flavonoid nucleus, the <sup>13</sup>C NMR (SEFT) spectrum of **1** exhibited seventeen carbon resonances belonging to three sugar moieties, and seven carbon signals indicating the presence of one acyl group. An additional signal at  $\delta_{\rm C}$  57.6 indicated a methoxylated flavonoid. Accordingly, the <sup>1</sup>H NMR spectrum showed three anomeric protons resonating at  $\delta_{\rm H}$  5.76 (d, J = 8.7 Hz), 5.48 (brs) and 4.59 (brs), corresponding in the HSQC spectrum to three anomeric carbons. The number of carbons together with the MS data indicated the presence of two hexoses and one pentose in the structure. In particular, the broad singlet at  $\delta_H$  5.48 correspoding to the anomeric carbon at  $\delta_C$  109.9 together with a quaternary oxygenated carbon at  $\delta_C$  80.2 and two methylenes at  $\delta_C$  75.8 and 70.9 were typical of an apiofuranose unit, whereas the broad singlet at  $\delta_{\rm H}$  4.59 (corresponding to the carbon at  $\delta_{\rm C}$ 102.4) and a methyl group at  $\delta_{\rm H}$  1.17 revealed the presence of an  $\alpha$ -rhamnose. COSY experiment together with HSQC enabled the complete characterization of the two sugars, which was further confirmed in an HSQC-TOCSY experiment. The latter one is of great importance for the structure elucidation of oligosaccharides [12], as it reveals in detail the sequences of all proton sugars belonging to individual saccharides. In this case it enabled the accurate characterization of the rhamnose group. Signal assignment of the third sugar showed it belonged to a galactose moiety, as indicated by a broad doublet (J = 3.3 Hz) at  $\delta_{\rm H}$  3.81 which corresponded to proton H-4". HSQC-TOCSY experiment was again typical of a galactose sequence, as it displayed signals solely from H-1 to H-4 (Fig 2). The blocking of magnetization transfer from the anomeric proton at H-4 of galactose has been described previously [13], and it is attributed to the particularly small scalar coupling constant (<1 Hz) between H-4 and H-5 in galactose. Under the experimental conditions used (100 msec) HSQC-TOCSY permitted the correct assignment of the galactose protons H-2" (dd, J = 9.5, 8.0 Hz), H-3" (dd, J = 9.7, 3.8 Hz) and H-4" (brd, J = 3.3 Hz) along the HSQC dimension of C-1" (Figure 2)

The lowfield shift of the methylene carbon of galactose at  $\delta_C$  67.7 suggested that this was one of the glycosylation sites. This was confirmed by interpretation of the HMBC spectrum. An HMBC crosspeak between the galactose H-1" and C-3 (at  $\delta_C$  134.5) proved that the saccharidic unit was attached to the position 3 of isorhamnetin, whereas diagnostic crosspeaks (Table 1) between H-2"/C-1"" and H-1"'/C-6" proved the 1 $\rightarrow$ 2 linkage of apiose and 1 $\rightarrow$ 6 linkage of rhamnose on galactose, respectively. In the aromatic area of the <sup>1</sup>H NMR spectrum remained signals which belonged to an AA'BB' system: four protons appeared as doublets at  $\delta_H$  7.53 (2H, J = 8.7 Hz, B2,6), as well as  $\delta_H$  6.61 (2H, J = 8.7 Hz, B3,5) and were assigned to a *p*-hydroxybenzoyl group.

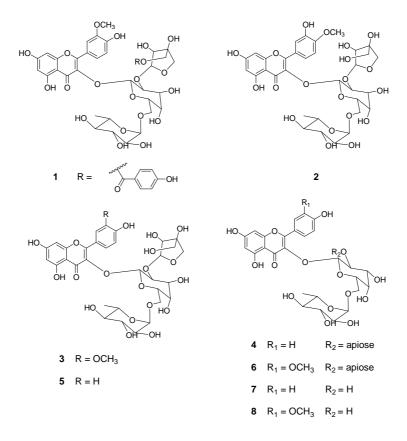


Figure 1. Structures of compounds (1-8)

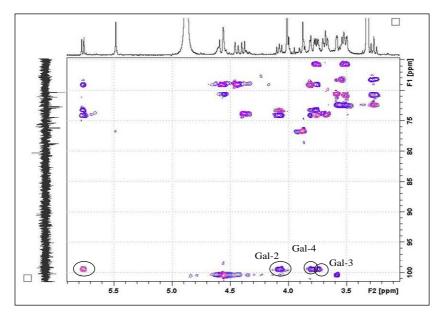


Figure 2. HSQC-TOCSY spectrum (in blue) overlapped with the HSQC spectrum (in red) of the sugar area of compound 1 (astrarmatuside)

The linkage of the *p*-hydroxybenzoyl group on the apiose was deduced from the downfield shifted signals of H-5a"", and H-5b"", (at  $\delta_H$  4.57 and 4.44, respectively). Additionally, the attachment of the acyl group to apiose was confirmed by HMBC crosspeaks between B8/H-5a"", and B8/H-5b"". Due to the vicinity of the acyl group to the C-5"" of apiose, ROESY crosspeaks were observed between B2,6 and H-5a"" & 5b"". The same spectrum exhibited crosspeaks between H-1", H-2', and H-

6', whereas interactions between the methoxyl group and H-2' confirmed the structure of isorhamnetin (Table 1).

C/H	$\delta_{\mathrm{H}}$	$\delta_{ m C}$	C type	HMBC
GENIN				
2	-	158.3	С	H2', H6'
3	-	134.9	С	H1"(Gal)
4	-	-	C=O	-
5		163.8	С	H6
6	6.08 d (J = 2.1)	101.4	CH	C5, C7, C10
7	-	166.1	С	H6, H8
8	6.17 $d$ (J = 2.1)	95.0	CH	C7, C9, C10
9	_	158.8	С	H8
10	_	106.4	С	H6, H8
-OCH <sub>3</sub>	4.01 <i>s</i>	57.6	CH <sub>3</sub>	C3'
1'	_	123.6	C	H2', H5'
2'	8.04 $d$ (J = 1.9)	115.0	СН	C2, C1', C3', C4
3'	-	148.9	C	OCH3
3 4'	-	150.9	C	H2', H5', H6'
5'	6.90 d (J = 8.4)	116.3	СН	C1', C3', C4'
6'	$7.36 \ dd \ (J = 8.4, 2.0)$	123.6	СН	C2, C4'
0	7.50 uu (5 – 0.1, 2.0)	125.0	en	02, 04
C/H				
galactose				
1"	5.76 <i>d</i> (J = 7.9)	101.4	СН	C3
2"	$4.08 \ dd \ (J = 9.5, 8.0)$	75.2	СН	C1""
2	+.00  uu  (3 - 9.5, 0.0)	15.2	en	CI
3"	3.76 <i>dd</i> (J = 9.7, 3.8)	76.04	СН	
4"	3.81 br <i>d</i> (J = 3.3)	71.0	СН	
5"	3.76*	75.97	СН	
ба"	3.76*	67.7	$CH_2$	
6b"	3.52*			
rhamnose				
1'''	4.59 brs	102.4	СН	C6"
2'''	3.58 <i>dd</i> (J = 3.1, 1.6)	72.6	СН	
3'''	3.51*	72.8	СН	
4'''	3.26 t (J = 9.6)	74.3	СН	
5"'	3.54*	70.2	СН	
6'''	1.17 d (J = 6.3)	18.4	CH <sub>3</sub>	
Apiose			2	
1''''	5.48 brs	109.9	СН	
2''''	3.88 brs	78.6	СН	
3''''	-	80.2	С	H5b'''', H4b''''
4a''''	4.38 d (J = 9.6)	75.8	CH <sub>2</sub>	, -
4b''''	3.70*		2	C3''''
5a''''	4.57 d (J = 11.7)	70.9	$CH_2$	B7 (C=O benzoy
5b''''	4.44 d (J = 11.1)		- 2	C3""
benzoyl group				
B1	-	123.8	С	B3,5
B2,6	7.53 d (J = 8.7)	132.9	СН	B6,2
B2,0 B3,5	6.61 d (J = 8.7)	116.3	СН	B5,3
	0.01  a  (3 - 0.7)	164.4	C	B2,6, B3,5
B4				

**Table 1.** <sup>1</sup>H (295 K, 400 MHz) and <sup>13</sup>C NMR (295 K, 100.6 MHz) Spectroscopic Data of Compound 1 (in CD<sub>3</sub>OD;  $\delta$  in ppm, *J* in Hz) and diagnostic HMBC cross peaks

\* signal pattern unclear due to overlapping

#### 3.1. Compound 1 (astrarmatuside)

Isorhamnetin-3-*O*-(5<sup>'''</sup>-*p*-hydroxybenzoyl)- $\beta$ -apiofuranosyl-(1 $\rightarrow$ 2)[ $\alpha$ -rhamnopyranosyl (1 $\rightarrow$ 6)]- $\beta$ -galactopyranoside (1) (astrarmatuside): Amorphous pale powder (3.9 mg); UV  $\lambda_{max}$  (MeOH): 255, 330 (nm); HR-ESI-MS (neg.) *m/z*: 875.22384 ± 0.24ppm  $\Delta$ mU (theoretical 875.22405, calculated for C<sub>40</sub>H<sub>43</sub>O<sub>22</sub>); diagnostic fragments: 737.19223 [M-benzoyl-H<sub>2</sub>O]<sup>-</sup>, 559.16531 [gal-rha-api-2H]<sup>-</sup>, 315 [A-H]<sup>-</sup>; <sup>1</sup>H and <sup>13</sup>C NMR spectral data, see Table 1.

# 4. Chemotaxonomic significance

From this study, We have separated eight flavonol glycosides from the endemic species *Astragalus armatus* from which compound (1), which we named astrarmatuside, is isolated for the first time from a natural source while compound (2) is reported here for the second time from nature; it was first isolated from *Milletia speciosa* and named millettiaspecoside D [14]. On the basis of a recently reported chemotaxonomic study of the genus [15], it appears that the compounds (4) [16] and (6) [17] are reported here for the first time for the genus while compounds (3) [18], (5) (mauritianin) [19], (7) (nikotiflorin) [20] and (8) (narcissin) [21] are isolated for the first time from the species.

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